Colony PCR

Colony PCR is useful in determining whether or not a specific colony on a plate has a sequence you desire. Primers for the specific sequence should be used when preparing the reaction cocktail. Examples of primers: antibiotic resistance, or primers flanking a cloned region.

A. Select colonies for analysis and amplify the cells

1. Select colonies to analyze and number them on the bottom side of the plate.
2. Prepare a 96-well plate and add 200ul LB solution with its resistance.
3. Pick up colonies by using toothpick and shake the colonies under 37°C for 2 hours.

B. Mix Colony PCR cocktail per the following recipe

Buffer (10X)	3.0uL
dNTPs (2.5 mM each)	0.6uL
Taq polymerase (5 units/uL)	0.15uL
Forward Primer	0.3uL
Reverse Primer	0.3uL
ddH2O	22.7uL
Colony	3uL
Total	30uL

C. Mix them together on ice.

D. Place the plate in the thermal cycle

Temperature Cycling:

94° C	2 min	
94° C	30s	30x
37 - 72° C	30 - 60 sec (anneal)	
72° C	30 - 60 sec (60 sec per kb target sequence)	
72° C	10 min	
4° C	Forever	

E. Run electrophoresis

E.